ZOOLOGICAL SCIENCE 8: 51–56 (1991)

Scanning Electron Microscopic Observations on the Wrinkled Blastula of the Sea Star, *Asterina minor* Hayashi

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ABSTRACT—Morphological changes during the wrinkled blastula stages were studied by scanning electron microscope in the sea star, *Asterina minor*. Wrinkling occurs from multiple invaginations of the blastoderm about 8 hr after fertilization. After the peak of the wrinkled stage, when the embryo is composed of an intricately folded sheet of blastomeres, the invaginations become shallower and decrease in complexity. Before the complete return to a smooth surface, invagination for gastrulation takes place. Blastomeres are ovoid or cuboid in the early stage and become high columnar late in the wrinkled stage.

INTRODUCTION

The wrinkling phenomenon in sea star blastula was first observed more than a century ago [1]. However, not much attention was paid to this phenomenon at that time. The wrinkled blastula was first thoroughly described in the sea star, *Cribrella oculata* (=*Henricia sanguinolenta*) by Masterman in 1902 [2], although he did not utilize the term wrinkle or wrinkled blastula. He described the process of wrinkling as a process of egression, contrary to the fact that wrinkling is actually brought about by ingressions, not by egressions. Wrinkled blastula formation was then subsequently described in some detail in the sea stars, *Solaster endeca*, *Porania pulvillus* and *Astropecten aranciacus* [3–5].

Newth [6] reported the occurrence of the same phenomenon in the sea cucumbers, *Cucumaria saxicola* and *Cucumaria normani*, and named the embryo with cellular ingressions, the wrinkled blastula. Since then, the wrinkled blastula has been reported in a number of asteroids and some holothuroid species. Wrinkled blastula formation was also reported in a few species of echinoids, *Phyllacanthus parvispinus*, *Peronella japonica* and *Asthenosoma ijimai* [7–9]. Thus, wrinkled blastula formation is known in three existent classes of Echinodermata, if not in all species of each class.

These previous studies were concerned with external, and in some species, internal changes which were observed in sectioned materials. In the present study, the process of wrinkling, from the commencement of wrinkling to complete recovery, was observed by a scanning electron microscope in the sea star, *Asterina minor*. One notable advantage in using *Asterina minor* as a material is that this species gives rise to fertilized ova and successive development without artificial means, thus avoiding any possible artificial deformation in the developmental process.

MATERIALS AND METHODS

Adults of *Asterina minor* Hayashi were collected from Toyama Bay and Kushimoto (Kii Peninsula) before the breeding season. They were reared in aquaria in the laboratory at 20–23°C using filtered off sea water (salinity, 33‰). During the breeding season, many individuals assemble and deposit fertilized eggs in the substratum in the laboratory as well as in the field [10]. Eggs from an adult develop nearly synchronously. Developmental

Accepted August 16, 1990
Received July 20, 1990
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stages were checked using a binocular dissecting microscope and developing embryos, from the beginning of wrinkling to the commencement of gastrulation, were fixed with 2% OsO_4 in 50 mM Na-cacodylate buffer (pH 7.4), its osmolarity being adjusted by adding sucrose. The fixed eggs were dehydrated in an ethanol series and dried with a critical-point-dryer (Hitachi, HCP-2). They were observed with a scanning electron microscope (Hitachi, S-510) after being coated with gold-palladium (Hitachi, E101 Ion Sputter). In most cases, the fertilization membrane was removed before the critical-point-dryer step. The inner structure of the embryos was observed on fractured sections.

RESULTS

As described previously [10], the fertilized ova initiate the first cleavage 3 hr after fertilization and reach the 64-cell stage 6.5–7 hr after fertilization through total, equal and radial cleavage. Since the ovum of this species is yolk-rich, early blastula (64～128-cell stage) has a very narrow blastocoel and the blastoderm is composed of ovoid blastomeres. Figure 1(A, B) shows an arrangement of blastomeres on the surface and inside at this stage.

Seven hr after fertilization, the embryos enter the wrinkled blastula stage. Cell division in the blastomeres on the surface of the embryo becomes irregular (Fig. 2A). At this stage, some blastomeres are apparently located in the blastocoel (Fig. 2B). Then the surface of the embryo becomes rapped due to the irregularity in blastomere arrangement (Fig. 3A) and the blastocoel is loosely filled with invaginated blastomeres of ovoid form (Fig. 3B). It was previously reported that wrinkling commences 8 hr after fertilization [10]. However, in the present study the commencement or initial sign of wrinkling was observed to begin slightly earlier than 8 hr after fertilization.

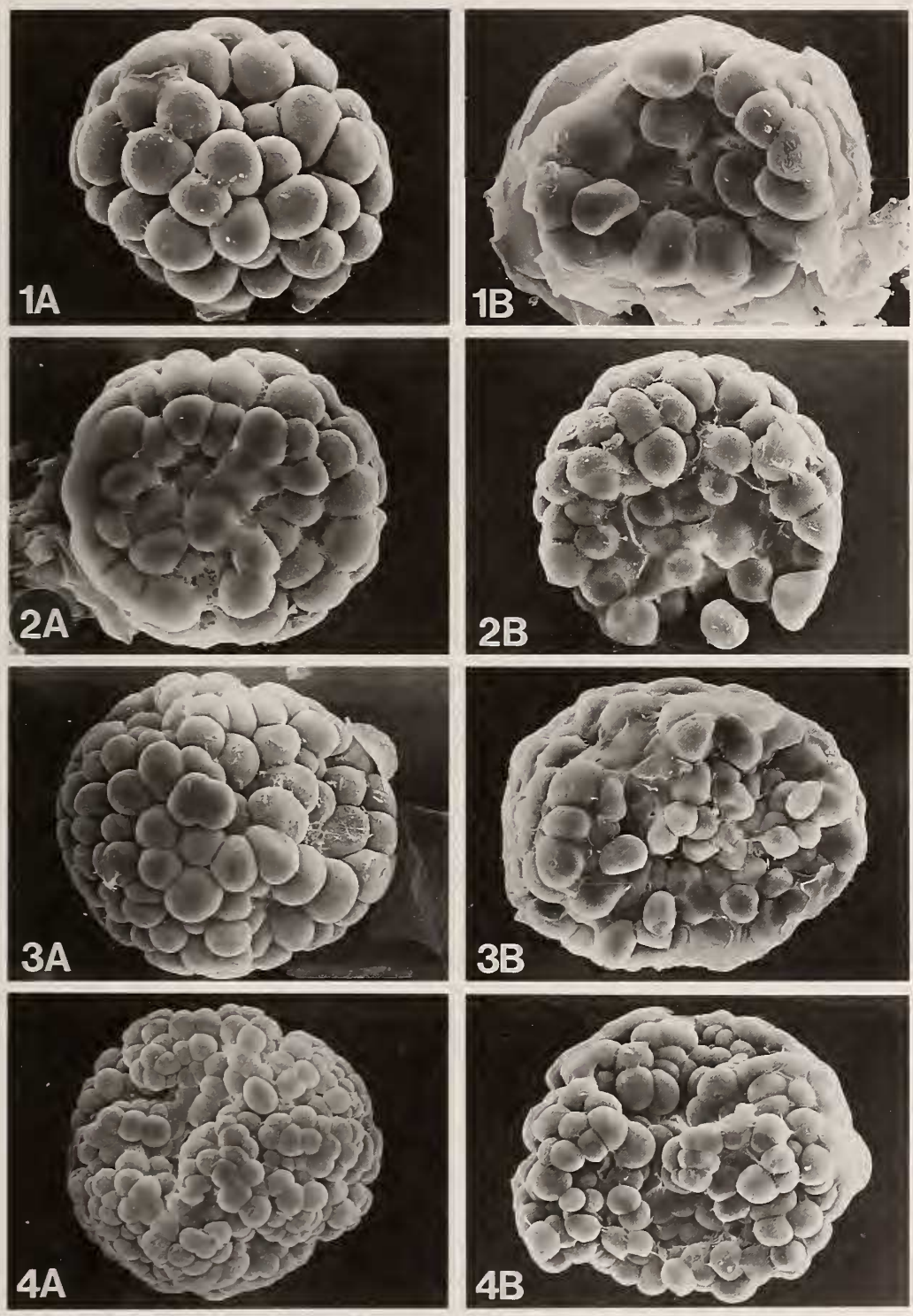
A little while later, the surface of the embryo is divided into small areas of cell clusters by furrows which are called egression grooves or egression tracts [4, 11] (Fig. 4A). Figure 4B shows the cell arrangement in the blastoderm at this stage. Subsequently, the egression tracts become deeper and more complex (Figs. 5A, 6A). Observations of the fractured sections show that the blastomeres in the blastocoel tend to be columnar (Figs. 5B, 6A, B) in contrast to ovoid or cuboid in the early stage of wrinkling (compare Figs. 5B, 6A, B with Fig. 4B). Twenty hr after fertilization, the blastomeres become tall columnar in shape and they are apparently arranged in a single layer (Fig. 7A). One notable characteristic among these stages is that the embryo is made up of an intricately folded cell sheet (Fig. 7A, B, C). This is the most wrinkled stage.

Then the furrows begin to decrease in complexity and become shallower. This is the process of the unfolding of the folded cell sheet. As a result of the unfolding, a space appears in the central portion of the embryo. However, as soon as the central space is formed, invagination for gastrulation occurs at the vegetal pole (Fig. 8A, B). Thus, the gastrula stage overlaps with the wrinkled blastula stage in the present species. Therefore, early gastrula bears many vestiges of the egression tract on the surface and even on the archenteron (Fig. 9A, B).

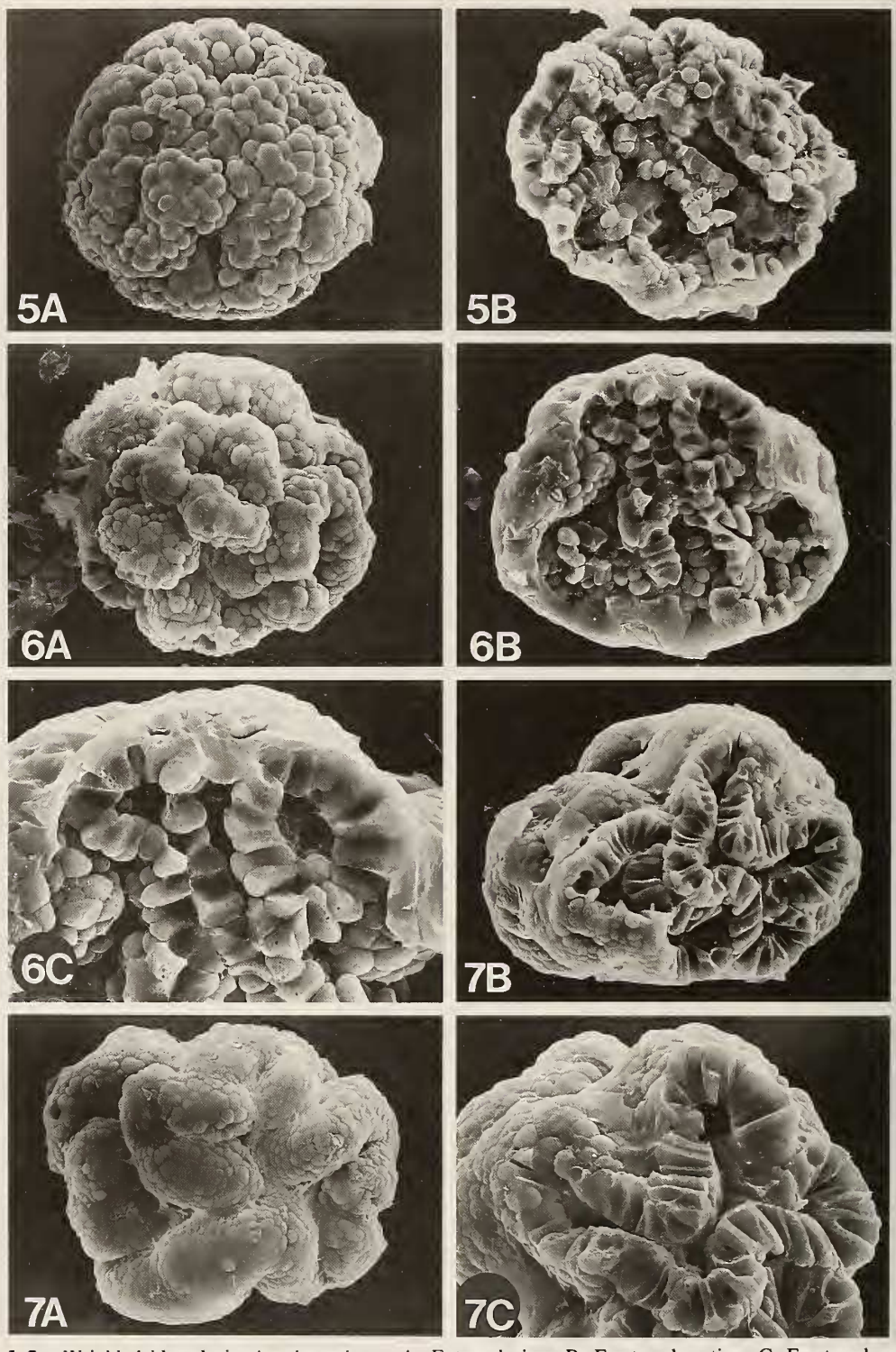
It is to be noted that the size (diameter) of the embryo does not change throughout the wrinkled blastula stage. In parallel with the progress of gastrulation, the vestiges of the egression tract decrease. Figure 10 (A, B) shows a gastrula from which the vestiges of the egression tracts have almost disappeared. Thereafter, gastrulation proceeds and the embryo becomes a typical gastrula as described previously [10].

DISCUSSION

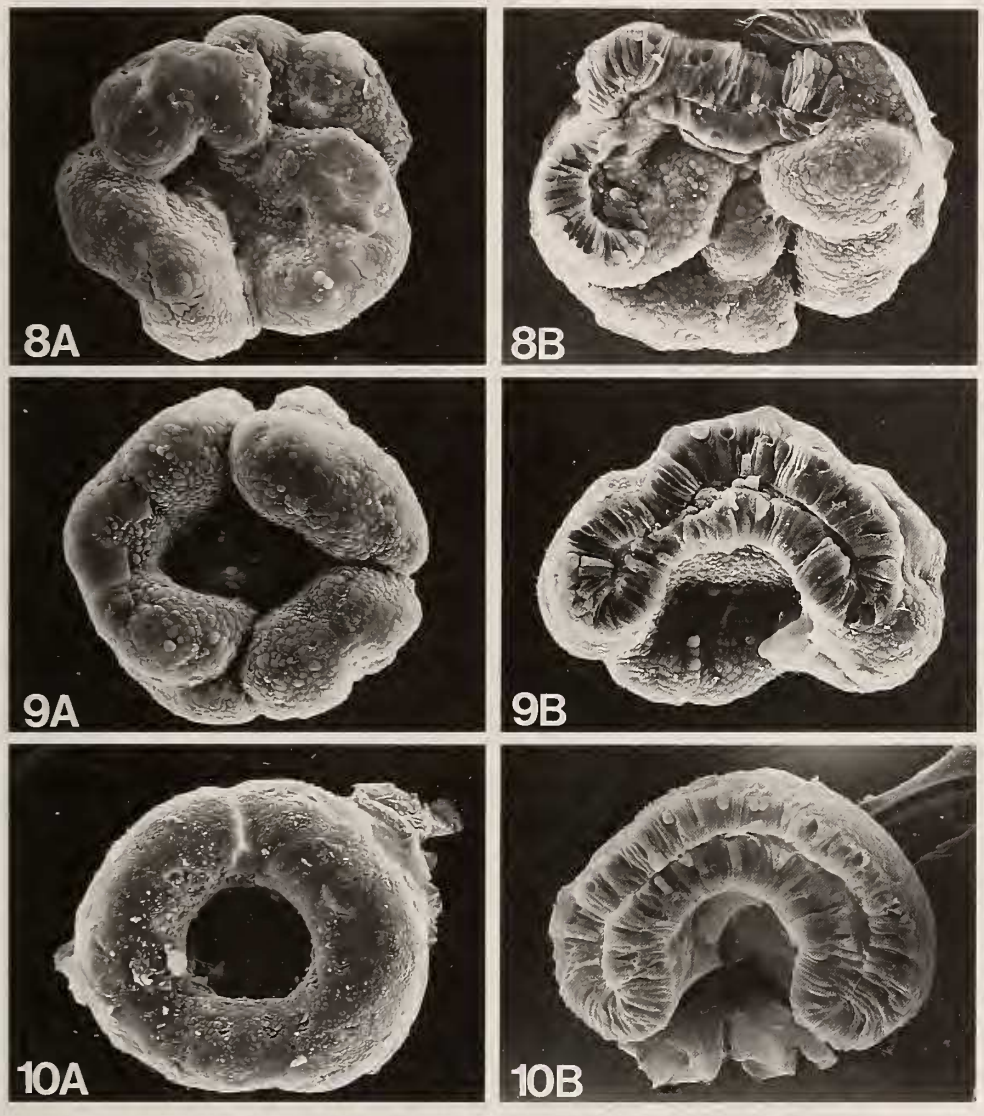
Since the detailed description of wrinkled blastula formation was given in the sea star, *Henricia sanguinolenta* by Masterman [2], this process has been reported in some species of Holothuroidea and Echinoidea and many species of Asteroidea. The occurrence of wrinkled blastulae is known in species belonging to all orders of Asteroidea whose development has been studied. The following lists some representatives of species having wrinkled blastula in each of these orders: Platyasterida, *Luidia quinaria* [12]; Paxillosida, *Astropecten polyacanthus* [13]; Valvatida, *Certonardoa semiregularis* [14]; Spinulosida, *Asterina coronata*



FIGS. 1–4. Wrinkled blastula formation in *Asterina minor*. A, External view; B, Fractured section. Detailed explanation in the text. 1 A, B. 64-128 cell-stage. 2 A, B. Seven hr after fertilization, before the commencement of wrinkling. 3 A, B. Eight hr after fertilization, beginning of wrinkling. 4 A, B. Ten hr after fertilization.



Figs. 5–7. Wrinkled blastula in *Asterina minor*. A, External view; B, Fractured section; C, Fractured section, enlarged picture. Detailed explanation in the text. 5 A, B. Fourteen hr after fertilization. 6 A, B, C. Sixteen hr after fertilization. 7 A, B, C. Eighteen hr after fertilization. The most wrinkled stage.



FIGS. 8–10. Wrinkled blastula and gastrula in *Asterina minor*. Detailed explanation in the text. A, External view (from vegetal pole); B, Fractured section (through animal-vegetal pole plane). 8 A, B. Beginning of invagination for gastrulation, 22 hr after fertilization. 9 A, B. Early gastrula with traces of egression tracts, 23 hr after fertilization. 10 A, B. Early gastrula with nearly smooth ectodermal surface, 25 hr after fertilization.

japonica [15]; Forcipulatida, *Leptasterias hexactis* [16]. This shows that the occurrence of the wrinkled blastula has no relation to the systematic position of the species.

The smallest ova developing through the wrinkled blastula is 125 μm in diameter in *Luidia quinaria* [12] and the largest is 1,000–1,200 μm in *Mediaster aequalis* [17]. Ova of many sea star species in the intermediate size between the two extremes have been known to pass through the wrinkled blastula stage during development. Thus, the size of ova also seems to have no bearing on the occurrence of the wrinkled blastula stage in asteroids. In contrast, the occurrence of wrinkled blastula in echinoids and holothuroids has been known in species having large-sized ova [6–9, 11].

From these facts, it is concluded that wrinkled blastula formation is not a rare, but a fairly common phenomenon is sea stars. In the present species, *Asterina minor,* fertilization occurs naturally, without any artificial means. This and the common occurrence of wrinkled blastula formation excludes the possibility that the wrinkled blastula stage is an artifact or an abnormal condition during development. Therefore, the wrinkled blastula stage should be examined by means of experimental or analytical developmental techniques. It is highly probable that many biochemical, morphological and/or kinetic changes occur during this stage. In fact, it was reported that the stainability and size of the blastomeres on the surface are different from those in the inside in *Asterina pectinifera* and *Astropecten scoparius* [18, 19].

It was observed in the present study that the size (diameter) of the embryo did not change throughout the wrinkled blastula stage. Blastomeres at the beginning of the wrinkled blastula (Fig. 2A, B) are ovoid and this situation continues for the first several cleavages after the commencement of wrinkling. As the cell divisions proceed and the number of blastomeres increases, the blastomeres become columnar (Figs. 6B, 7B), eventually becoming very high columnar (Fig. 8B).

In the present study, the details of the changes in cell shape and the process of multiple invaginations and the recovery were observed. This gives us a basic morphological knowledge on the process of wrinkled blastula formation.

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